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I also certify that by virtue of an assignment registered under the Patents Act 1977, the application is now proceeding in the name as substituted.

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Signed R. Mahoney

Dated 30 April 1999

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9805699.7.

By virtue of a direction given under Section 32 of the Patents Act 1977, the application is proceeding in the name of

Cambridge Biostability Limited
School of Applied Sciences
Anglia Polytechnic University
East Road
Cambridge
CB1 1PT.

GB9805699.7

By virtue of a direction given under Section 30 of the Patents Act 1977, the application is proceeding in the name of

Eastbridge Limited
4 Archway Court
Barton Road
Cambridge
CB3 9LW

[ADP No. 07270531001]

Patents Form 1/77

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Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form.)

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Fee: £25

1.	Your reference	18 MAR 1998 39567/JMD
		19MAR98 E344497-1 D01431 P01/7700 25.00 - 9805699.7
2.	Patent application number *(The Patent Office will fill in this pa*	9805699.7
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	ANGLIA RESEARCH FOUNDATION Anglia Polytechnic University, East Road, Cambridge CB1 1PT.
	Patents ADP number *(if you know it)*	SECTION 30 (1977 ACT) APPLICATION FILED 16.7.98. 7177314001
	If the applicant is a corporate body, give the country/state of incorporation	United Kingdom
4.	Title of the invention	NEW STABILISING GLASSES
5.	Full name, address and postcode in the United Kingdom to which all correspondence relating to this form and translation should be sent	Reddie & Grose 16 Theobalds Road LONDON WC1X 8PL
	Patents ADP number *(if you know it)*	91001

		Country	Priority application *(if you know it)*	Date of filing *(day/month/year)*
6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number			

		Number of earlier application	Date of filing *(day/month/year)*
7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application		

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
   a) any applicant named in part 3 is not an inventor, or
   b) there is an inventor who is not named as an applicant, or
   c) any named applicant is a corporate body.
   See note (d))

   YES

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9. [redacted]r the number of sheets for any of the [redacted]wing items you are filing with this form. [redacted] not count copies of the same document.

Continuation sheets of this form	
Description	5
Claim*(s)*	2
Abstract	0
Drawing*(s)*	2 RC

10. If you are also filing any of the following, state how many against each item.

Priority documents	0
Translations of priority documents	0
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	0
Request for preliminary examination and search *(Patents Form 9/77)*	1
Request for substantive examination *(Patents Form 10/77)*	0
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature: JMDavies for Reddie & Grose

Date: 18 March 1998

12. Name and daytime telephone number of person to contact in the United Kingdom

J M DAVIES
0171-242 0901

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Notes

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NEW STABILISING GLASSES

Drying of biological molecules such as foodstuffs, vaccines and drugs is an ancient way of preserving them. However, a price that usually has to be paid for increased stability is often a decrease in quality. More recently it has been learned that certain additives can dramatically improve the retention of the native properties of biological substances during drying. Levine and Slade were amongst the first to realise that the best additives were substances that tended to solidify from solution as an amorphous glass rather than by forming crystals (Levine H and Slade L., Principles of cryostabilisation technology from structure/property relationships of carbohydrate/water systems: A review Cryoletters 9 21-63 (1988)). Foremost amongst the stabilisers they recommended were certain sugars, notably sucrose. Although not formally proved, the stability of these dried formulations is assumed to be a function of the glass transformation of the sugar solution as it dries. As a consequence of the progressive increase in viscosity of the sugar syrup as it dries, it is thought that the active molecules are concentrated in imperceptibly small and hence essentially continuous steps from their mobile native state in liquid solution to an immobilised solid solution or glass. In the glass, molecular diffusion and hence molecular interactions are negligible. Thus the chemistry responsible for degradation is arrested and the product remains stable as long as it remains dry and glassy (Franks F., Freeze drying: From empiricism to predictability Cryoletters 11 93-110 (1990)).

This simple view that glass formation is the explanation for stability is incomplete. This explanation holds for glasses that are not inherently chemically reactive or unstable. It is incontrovertible that certain substances that form excellent glasses are nevertheless very poor stabilizers. Many of these are either chemically reactive or are unstable and break down to reactive intermediates and degrade the product during storage in the dry state (Newman Y.M., Ring S.G. and Colaco C. The role of trehalose and other carbohydrates in biopreservation Biotechnol. & Genet. Eng. Rev.

11 263-294 (1993)). A common reactivity of sugar glasses is the reducing action of the carbonyl group and the degradation products formed are often the familiar carbonyl-amine compounds of the Maillard reaction (Ellis G.P. The Maillard reaction. Adv. Carbohyd. Chem. 14 63-134 (1959)). Because the Maillard reaction is temperature dependent, it is only slowly progressive at low temperatures. Many glass-forming materials give excellent preservation of activity during the drying process itself but the product subsequently undergoes progressive deterioration unless stored under refrigeration.

The error of ignoring the subtle sugar chemistry that can proceed in dried preparations is widespread in the literature and has led to the advocacy of simple tests of the glass transition temperature of pure sugar solutions as a means of selecting good stabilisers. This approach has actually led to the recommendation of quite useless substances in the past (Franks F. Freeze drying: From empiricism to predictability Cryoletters 11 93-110 (1990)). Rather, the efficacy of a formulation is the result of physical and chemical interactions between the multiple components in the formulation on drying. All these interactions may not be predicted by current theories.

The superiority of the disaccharide trehalose as a stabiliser was first indicated by its prevalence in rare living creatures which regularly dried out and could come back to life on rehydration (Crowe J.H., Crowe L.M. and Chapman D. Preservation of membranes in anhydrobiotic organisms: The role of trehalose Science 223 701 (1984)). In laboratory studies trehalose incorporated into the buffer solutions from which active biomolecules were dried resulted in a product with quite remarkable resistance to denaturation by heat (Colaco C.A.L.S., Sen S., Thangavelu M., Pinder S. and Roser B. Extraordinary stability of enzymes dried in trehalose: Simplified molecular biology. Biotechnol. 10 1007-1011 (1992)). Because it is rapidly degraded in the body by a specific trehalose to two molecules of glucose, trehalose possesses many of the properties of the ideal industrial stabiliser for foods and medical products.

A large scientific and patent literature has now developed on trehalose stabilisation of foods, vaccines, diagnostics and drugs. The disadvantages of trehalose are its expense and the presence of contaminating reducing sugars, especially glucose, in all but the most rigorously purified material.

One of the non-reducing and chemically stable sugar derivatives that might be expected to stabilise effectively is the non-hygroscopic monosaccharide, mannitol. Because of its remarkable resistance to water sorption at high atmospheric humidity (Wade A. and Weller P.J. Handbook of Pharmaceutical Excipients second edition p296 (1994)), it is widely used in tablet and powder formulations as a bulking and anti-caking agent. In combination with other excipients such as glycine, it is also widely used in freeze dried parenteral preparations but is added merely as a carrier to produce a stiff, homogeneous cake that improves the appearance of the lyophilised plug in a vial (Wade & Weller 1994).

In published surveys of the stabilising ability of a wide range of sugars and sugar derivatives (Colaco C.A.L.S., Smith C.J.S., Sen S., Roser D.H., Newman Y., Ring S. and Roser B.J. Chemistry of protein stabilisation by trehalose in "Formulation and delivery of proteins and peptides" Cleland and Langer eds American Chemical Society Washington 222-240 (1994)), it was shown that mannitol and sorbitol were very poor stabilisers. Indeed in several patent applications it has previously been claimed that mannitol and certain other monosaccharide alcohols cannot stabilise at all. PCT published application No WO 91/18091. "Stabilisation of biological macro-molecular substances and other organic compounds", Roser B.J. and Colaco C. priority date 14 May 1990 claimed that only non-reducing glycosides of a polyhydroxy sugar alcohol or other straight chain polyalcohol or raffinose, stachyose or melezitose would work as stabilisers especially on storage. This patent states "Thus the monosaccharide sugar alcohols galactitol, mannitol and erythritol are not satisfactory protective agents". US patent Number 5,621,094 "Method of Preserving

Agarose Gel Structure During Dehydration by Adding a Non-reducing Glycoside of a Straight Chain Sugar Alcohol" Roser B. and Colaco C. with the same priority date shows that "glucose mannitol and sorbitol failed after one week" while "lactitol and trehalose were perfect after >12 weeks" and further defined effective formulations as "wherein the non-reducing glycoside of a straight chain sugar alcohol does not form crystals during dehydration". Again PCT published application No WO 96/05809 "Improved method for stabilisation of biological substances during drying and subsequent storage and compositions thereof" Colaco C. Roser B.J. and Sen S. Priority date 19 August 1994 claims methods wherein even reducing sugars are proposed to be used to stabilise products by preventing them from attacking the product by means of Maillard reaction inhibitors, but the application states that mannitol has no stabilising effect whatsoever.

We have now found this to be incorrect. In contradiction to the statements in these documents we have found that certain monosaccharide sugar alcohols such as mannitol and inositol can be excellent stabilisers when correctly formulated and in fact have significant advantages over trehalose for many applications. In view of mannitol's acceptance by regulatory authorities and widespread use in the healthcare industry in both parenteral and oral formulations, it has considerable advantages as a new stabilising excipient. Its low cost and chemical inertness, together with its exceptional stability and its high purity and safety, would make it the stabiliser of choice for pharmaceuticals. We have found that certain substances must be added to a formulation to convert mannitol into an excellent stabiliser. The effect of these additives is dose dependent and below a threshold concentration they do not work. The compounds useful as additives in accordance with this invention promote the drying of mannitol solutions as glasses rather than crystals. One of the most potent additives is the borate ion either as boric acid, or tetraborate salts of sodium or potassium. This probably forms a network complex with mannitol or even a covalent compound, sodium mannitoborate. Other effective materials such as calcium lactate,

and proteins such as serum albumin, or polyamine materials such as polyvinyl pyrollidone, or polyvinyl alcohol, intrinsically form glasses when dried from solution. Yet other effective additives such as acetate salts, will form glasses themselves but only when quenched from the melt and only when the melt contains several metal cations rather than a single cation, such as sodium and calcium. An additional property of the materials identified to date is that the beneficial actions of these materials are additive so that they can be mixed together in successful formulations which contain sub-threshold doses of each additive alone. Other additives which are either themselves glass-formers (under certain conditions) or are glass-formation-facilitators such as the phosphate salts of sodium and potassium and sodium silicate are capable of being utilised to make stabilising glasses according to this invention.

A number of other sugar derivatives previously rejected as stabilising agents in the prior art listed above such as xylitol, inositol and also stabilise very effectively when correctly formulated so as to promote the formation of a stabilising glassy matrix, rather than crystals, on drying.

The quality of the glasses made by this process is high. The glass transition temperature (Tg) of 1:1 w/w mannitol/calcium lactate glass is around 68°C (Figure 1). This compares with a Tg of around 90°C for a trehalose/sodium sulphate glass dried under the same conditions (Figure 2). Both types of glass have Tg's well above any possible ambient storage temperature and , because the glasses are chemically inert and non-reactive, the entrapped products are stable at room temperatures and require no refrigeration of any kind.

It is a particular advantage of this invention that sugars which have previously been known as stabilisers in methods of freeze drying may be used successfully in drying compounds subject to deactivation on drying whilst utilising drying temperatures above freezing point (e.g. room temperature or above).

Claims

1. A method of drying a compound which is subject to deactivation on drying, or a mixture of such compounds, comprising subjecting an aqueous system containing the compound or mixture to drying in the presence of a monosaccharide sugar alcohol and at least one additive which is a glass-former or a glass-formation-facilitator, whereby the compound solidifies from solution as an amorphous glass rather than by forming crystals.

2. A method according to claim 1 in which the aqueous system contains from 0.05 to 90% by weight of sugar alcohol.

3. A method according to claim 1 in which the ratio of sugar alcohol to compound is at least 0.5:1 by weight.

4. A method according to any of claim 1 to 3 in which the compound is a protein, polysaccharide or nucleic acid.

5. A method according to claim 4 in which the compound or mixture comprises an enzyme, serum, serum complement, an antibody or antigen (either free or coupled to a support), a nucleic acid, a fluorescent protein, or a vaccine component.

6. A method according to any of claim 1 to 5 in which the system is dried under conditions selected from one or more of the group consisting of ambient temperature or above, freeze drying, spray drying, vacuum drying and drying at atmospheric pressure.

7. A dried product which is an amorphous glass containing monosaccharide sugar alcohol and at least one additive which is a glass-former or a glass-formation-

facilitator and a compound which is subject to deactivation on drying, or a mixture of such compounds in a weight ratio of at least 0.5:1 respectively, the product having been dried.

8. A dried product according to claim 7 in which the compound is a protein, polysaccharide or nucleic acid.

9. A dried product according to claim 8 containing an enzyme, serum complement, an antibody or antigen (either free or coupled to a support), a nucleic acid, a fluorescent protein, or a vaccine component.

10. A method or product according to any preceding claim wherein the sugar alcohol is selected from the group consisting of mannitol, galactitol, xylitol rabitol, arabitol and inositol.

11. A method or product according to any preceding claim wherein there is one or a mixture of additives selected from the group consisting of protein, borate ion, calcium lactate, phosphate, silicate and acetate salts.

12. A method or product according to claim 11 wherein at least one additive is selected from the group consisting of boric acid, tetraborate salt of sodium or potassium and sodium mannitoborate.

Figure 1 MANNITOL / CALCIUM LACTATE 1 : 1

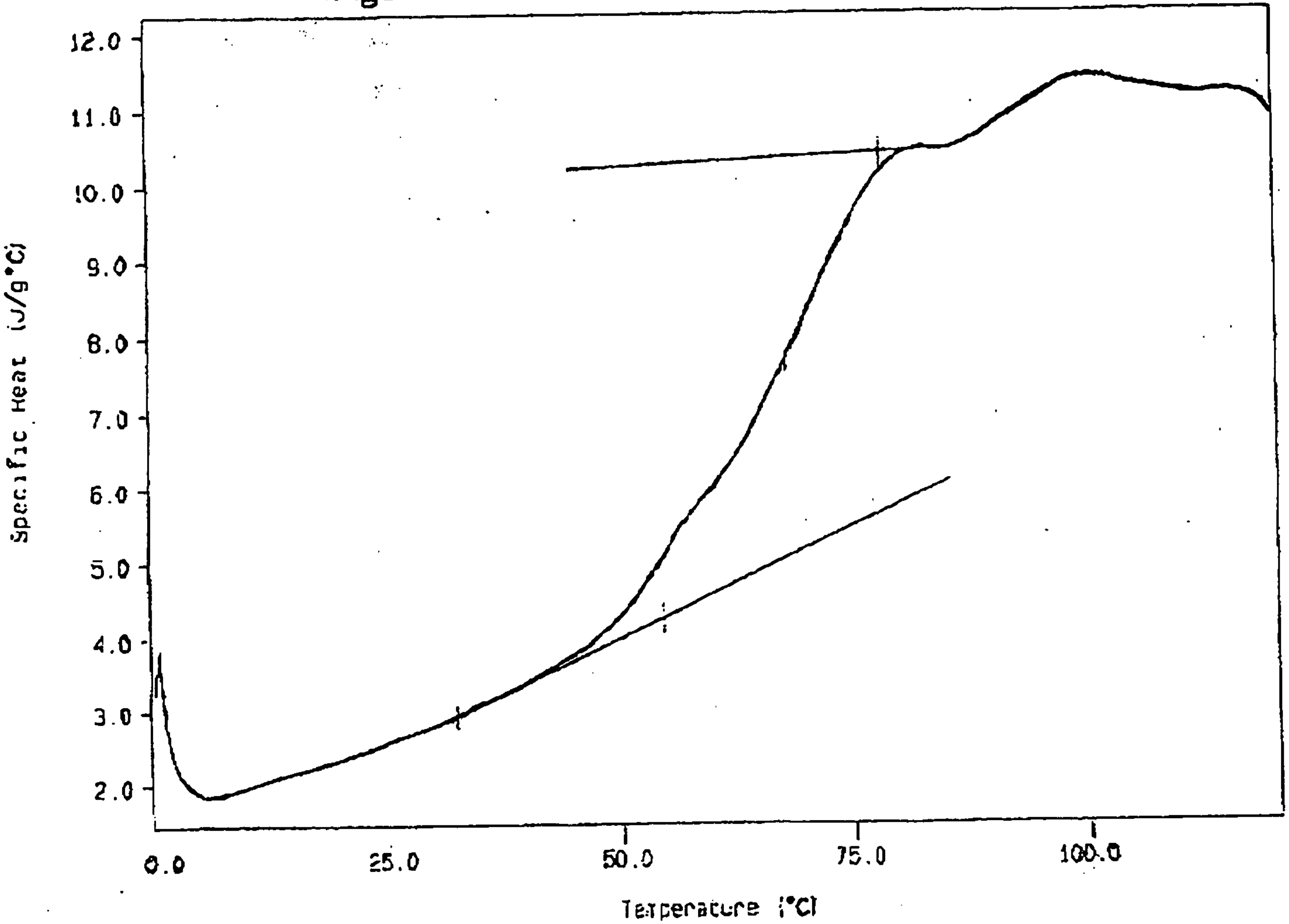

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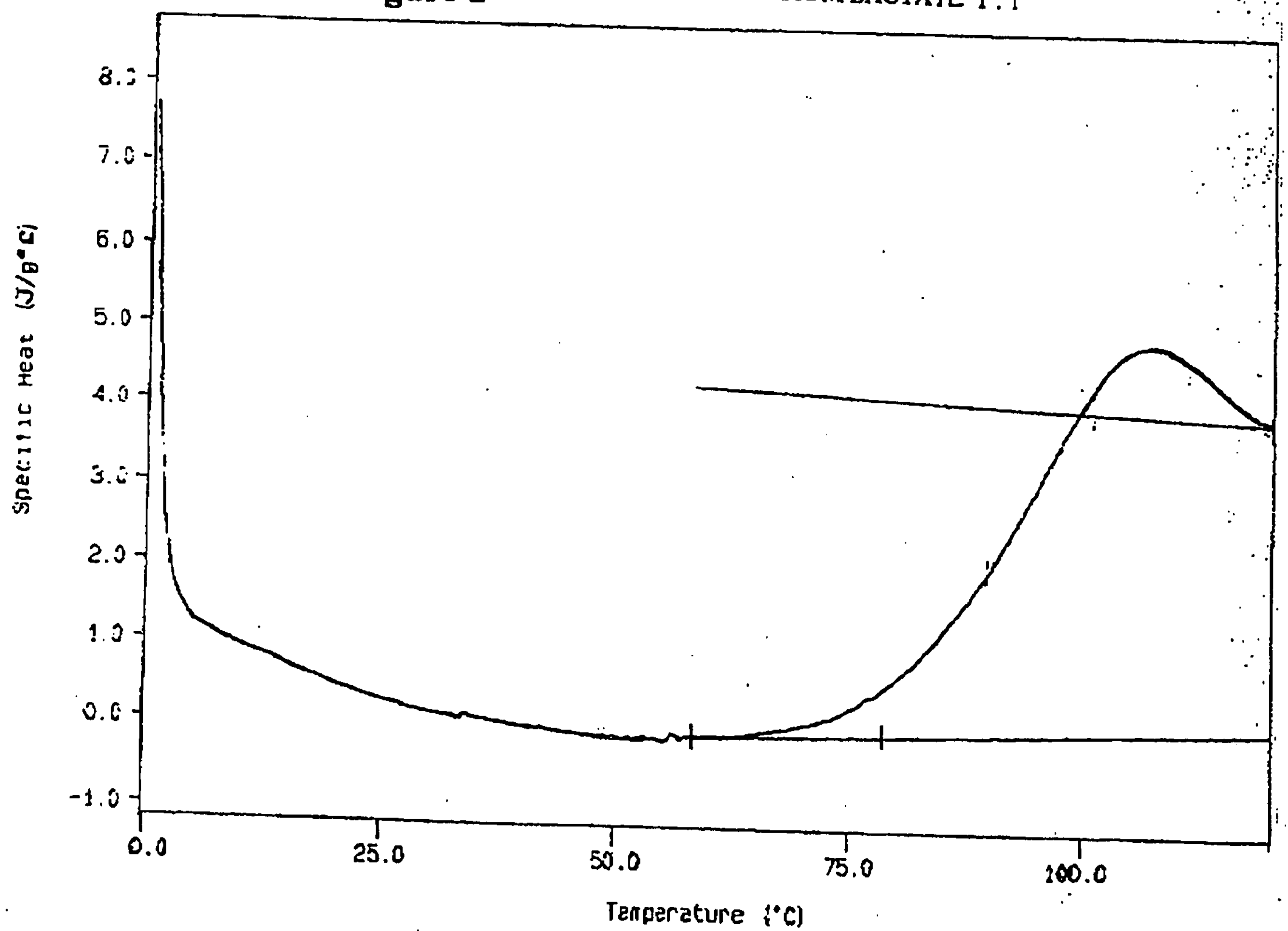
Figure 2 TREHALOSE / CALCIUM LACTATE 1 : 1



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